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Oestrogen replacement therapy may improve memory functioning in the absence of APOE $\varepsilon 4$

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Abstract. There is currently intense controversy regarding the use of hormone replacement therapy (HRT) in postmenopausal women, in relation to its therapeutic efficacy in Alzheimer's disease (AD). It has been suggested that the benefits of HRT may be modified by apolipoprotein E (APOE) genotype (the major genetic risk factor for AD). Here we report the findings of the first study designed to systematically explore the interaction of (a) oestrogen replacement therapy (ERT) and (b) possession of an $\varepsilon 4$ allele of APOE on specific elements of episodic learning and memory that are commonly used indices of age-related cognitive decline. This data represents a cross-sectional analysis of the interaction of ERT and APOE genotype on learning and memory in a cohort of 181 healthy postmenopausal women [ERT users ($n = 101$, mean age 65.40 $\pm$ 6.34); ERT non-users ($n = 80$, mean age 67.03 $\pm$ 6.80)] residing in Perth, Western Australia. The highest level of learning (trials 2–5; $P < 0.05$) and memory (e.g. total number of items recalled; $P < 0.05$) performance was observed in women taking ERT who were not carriers of the APOE $\varepsilon 4$ allele. APOE $\varepsilon 4$ carriers receiving ERT performed no better on episodic memory testing than APOE $\varepsilon 4$ carriers who were not receiving ERT. These cognitive differences related to genetic profile, were noted on both recall and recognition ($P = 0.005$) tests of memory. The findings have significance for evaluating whether and when ERT may be clinically indicated. Specifically, ERT may benefit the cognitive functioning of women not carrying the APOE $\varepsilon 4$ allele.

Keywords: Oestrogen supplementation, post-menopausal women, cognition, apolipoprotein E genotype, Alzheimer's disease

1. Introduction

The use of hormone replacement therapy (HRT) in postmenopausal women is currently highly controversial, in particular with regard to the possibly increased incidence of breast cancer and cardiovascular disease [1]. In addition, there has been some suggestion that combined oestrogen-progesterone therapy may actually increase the risk of Alzheimer's disease

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if initiated at age 65 or older [2]. This paper reports the first study to systematically evaluate the effects on episodic learning and memory of unopposed-oestrogen replacement therapy (ERT) in women either possessing or lacking the ε4 allele of the apolipoprotein E (APOE) gene. APOE ε4 is thought to predispose individuals to the incidence of Alzheimer's disease (AD) [3–5]. Perhaps independently, APOE ε4 may be an important factor in modulating normal age-related cognitive change [6]. Furthermore, APOE ε4 has been suggested to confer a higher risk for AD in women (in whom there is a higher overall incidence of AD) compared to men [7–9].

A decrease in endogenous oestrogen levels after menopause has been proposed as a trigger for the development of AD in women [10,11]. A significant body of evidence suggests that HRT administered to postmenopausal women may prevent or delay the onset of AD [11,12] but neither treats nor reverses the disease [13,14]. In recent years researchers have therefore directed their attention towards evaluating the role of oestrogen in delaying and/or preventing the onset of age-related cognitive decline. Improvements have previously been observed following oestrogen supplementation in healthy postmenopausal women on tests of episodic learning and memory [15,16], and in some women with mild AD [17]. Interestingly, it has been suggested that the therapeutic efficacy of HRT is modified by APOE genotype [18,19]. Specifically, carriers of the ε4 allele do not seem to benefit from receiving oestrogen [19]. However, the possible interaction of ERT on episodic memory performance in those with or without an APOE ε4 allele has not yet been thoroughly investigated. This was the central goal of this study. We evaluated episodic verbal learning and memory, as deficits in these cognitive domains are apparent in the very early stages of AD [20].

2. Materials and methods

2.1. Participants

A total of 181 cognitively healthy Caucasian postmenopausal women were tested. Participants were currently enrolled in a longitudinal study on the effects of different forms of HRT on cognitive ageing (assessed via global measures of cognition) at the Sir James McCusker Alzheimer's Disease Research Unit at the University of Western Australia, Perth, Western Australia. To achieve the goal of this study, (a) a fresh selection criteria (outlined in detail below) was placed on the participants of the longitudinal study, to yield a subset consisting only of unopposed oestrogen takers (i.e. no progestin, either independently or in conjunction with oestrogen) and (b) to this subset a neuropsychological assessment testing the cognitive domain of interest (i.e. episodic learning and memory) had to be administered.

Participant information relating to medical history, use of ERT and recent performance on the Mini Mental Status Exam (MMSE) [21] and the Cambridge Examination for Mental Disorders in the Elderly- Cognitive Section (CAMCOG) [22] was available. Participants were selected for the study if they met the following criteria: (a) score $>$ 24 on the MMSE and (b) score $>$ 80 on the CAMCOG. Taken together, criteria (a) and (b) excluded individuals with dementia from the study. The application of these criteria therefore excluded individuals with frank cognitive deficits. In addition, (c) individuals with medical history of stroke, minor head injuries (e.g. concussion) and/or functional psychiatric disorders (e.g. clinical depression) were excluded from the study and (d) participants were further selected for the study on the basis of whether they were currently taking unopposed ERT. Specifically, participants were selected for the study if they were receiving one standard clinical dose of oestrogen (i.e. 0.625 mg Premarin or equivalent), and (e) if they had been taking ERT for more than 12 months. The rationale for employing these selection criteria (d) and (e) were as follows; (i) to negate dose to dose variability and in turn standardize dosages of different forms of ERT (i.e. Premarin, Ogen, Progynova, Transdermal Oestrogen and Oestradiol), and (ii) by excluding use under 12 months, participants included in this study were taking ERT for a sufficient duration for it to have therapeutic efficacy. The literature suggests that the efficacy of ERT in delaying the onset of preclinical cognitive decline and AD is both dose and duration dependent [9]. Finally, (f) individuals with no prior history of ERT use were selected as control participants [control participants underwent the same exclusion criteria (a) to (c) noted above]. Demographic characteristics of the cohort are presented broken down by ERT and APOE status, respectively (Tables 1 and 2).

This study was conducted in accordance with the guidelines of local institutional human research ethics committees (University of Western Australia and Murdoch University) and informed consent was obtained from all participants.

Table 1
Demographic Characteristics of the Cohort

	ERT users ($n = 101$)			ERT Non-users ($n = 80$)		
	Mean	SD	Range	Mean	SD	Range
Age (years)	65.40	±6.34	50–78	67.03	±6.80	54–80
Educationa	12.68	±2.74	8–22	12.43	±3.11	9–24
Baseline MMSEb	28.85	±1.35	25–30	28.98	±1.16	25–30
Baseline CAMCOGc	99.49	±4.17	85–106	99.36	±3.86	88–107

Note. [a]Values represent total years of education (years of schooling + years of higher education).
[b]Maximum possible score = 30.
[c]Maximum possible score = 107.

Table 2
Demographic Characteristics of the Cohort

	$\varepsilon4^+$ ($n = 64$)			$\varepsilon4^-$ ($n = 117$)		
	Mean	SD	Range	Mean	SD	Range
Age (years)	66.86	±6.63	52–79	65.71	±6.54	50–80
Educationa	12.16	±2.96	8–22	12.79	±2.86	9–24
Baseline MMSEb	28.69	±1.34	25–30	29.03	±1.21	25–30
Baseline CAMCOGc	99.13	±4.61	85–107	99.60	±3.68	89–106

Note. $\varepsilon4^+$, possession of an APOE $\varepsilon4$ allele; $\varepsilon4^-$, absence of the APOE $\varepsilon4$ allele.
[a]Values represent total years of education (years of schooling + years of higher education).
[b]Maximum possible score = 30.
[c]Maximum possible score = 107.

2.2. Neuropsychological assessment

Learning and memory of verbal information was assessed by administration of the California Verbal Learning Test (CVLT) [23], a multidimensional neuropsychological instrument that assesses episodic learning and memory (i.e. immediate and delayed recall and delayed recognition) within the context of an everyday memory task (i.e. remembering a word list). There is a 20-minute memory interval on the CVLT, after which delayed memory performance (recall and recognition) is evaluated. During this delay, the Beck depression inventory second edition (BDI-II) [24] was administered to participants (this score was obtained due to the known correlation between current depressive symptomatology and cognitive performance, which may be especially relevant in the elderly). Blood was also taken during the CVLT memory delay using a standard venipuncture technique.

2.3. APOE genotyping

Peripheral blood leukocyte DNA was extracted using standard protocols [25]. APOE genotyping was performed via PCR amplification as described previously [26]. The oligonucleotide primers were used as described elsewhere [27]. The amplified product was digested using the restriction enzyme *HhaI* (Fisher Biotech, Perth, Australia), followed by visualization under UV light to reveal DNA fragments with electrophoretic migration patterns unique to each allele [27].

3. Results

In this study the 4 groups of participants that were tested are represented by the following nomenclature: E^+ and E^- refer to whether or not participants in that group were receiving ERT at the time of testing, whereas $\varepsilon4^+$ and $\varepsilon4^-$ refer to whether participants in that group were carrying an $\varepsilon4$ allele.

3.1. Descriptive statistics

Due to the manner in which the sample was recruited there were a higher proportion of individuals carrying the $\varepsilon4$ allele than would be expected in the general population. However, there is no difference in the frequency of the $\varepsilon4$ allele between the E^+ and E^- groups (Pearson's χ^2 value = 0.05, $P = 0.82$). As such, any effect that this selection bias may have had would be expected to be consistent between the groups and thus negated. Additionally, the E^+ and E^- groups did not differ with respect to the following: demographic variables (i.e. age, educational level), overall cognitive

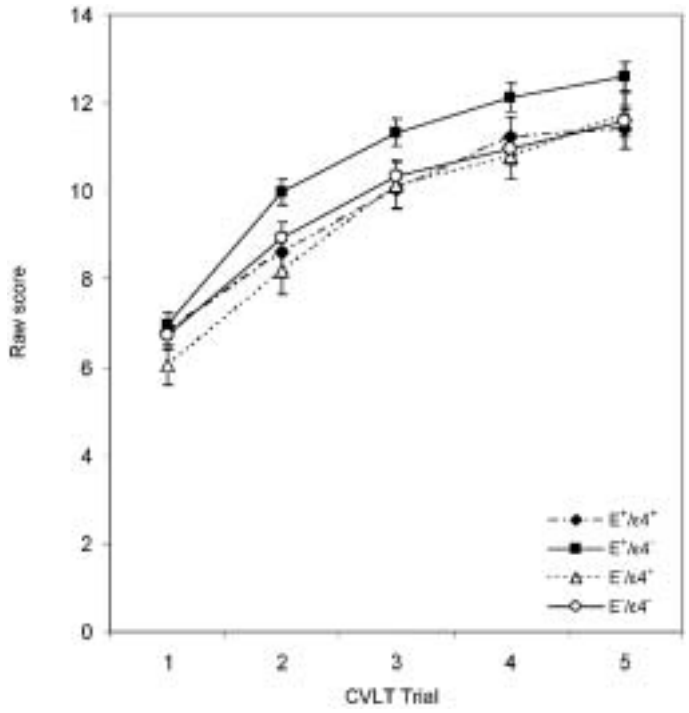

Fig. 1. Learning across the five CVLT trials as a function of participant group status. Error bars represent standard error of the mean. E^+ and E^- refer to whether or not participants in that group were receiving ERT at the time of testing. $\varepsilon 4^+$ and $\varepsilon 4^-$ refer to whether participants in that group were carrying an $\varepsilon 4$ allele. Note that there was no statistically significant difference between performances of the four groups on the first learning trial, but significant differences had emerged by the second trial.

status (i.e. score on the CAMCOG and on the MMSE) (Table 1) depression scores on the BDI-II, and family history of AD.

The $\varepsilon 4^+$ and $\varepsilon 4^-$ groups did not differ with respect to demographic variables, overall cognitive status (Table 2) or depression scores. The use of ERT in the $\varepsilon 4^+$ and $\varepsilon 4^-$ group was similar. However, as anticipated, a family history of AD was significantly higher in individuals identified as possessing the APOE $\varepsilon 4$ allele compared to the non-carriers (Pearson's χ^2 value $=$ 4.60, $P = 0.03$).

From the results of the cognitive assessment that was administered to participants, the following variables were highly correlated with all of the learning and memory parameters that were assessed: (a) age and scores on (b) the CAMCOG and on (c) the MMSE. These variables (a), (b) and (c) were therefore used as covariates in the statistical analyses of the memory data that are reported below.

The learning and memory data are summarized in the next paragraph, and then presented in more detail in subsequent paragraphs.

3.2. Learning and memory data

Better cognitive performance across the learning and memory trials was observed with ERT treatment in individuals who were not carrying the APOE $\varepsilon 4$ allele, but not in individuals who were carrying the $\varepsilon 4$ allele, with the $E^+/\varepsilon 4^-$ group performing best overall. This effect was apparent on both recall memory and recognition memory. These overall findings are presented in more detail below (Fig. 1 and Table 3).

3.2.1. Learning

A 2-way (4 $\times$ 5: group $\times$ learning trial) ANCOVA indicated that there was a trend towards a significant difference in the level of performance between the four participant groups when the recall data were collapsed across learning trials 1–5, F (3,174) $= 2.13$, $P = 0.10$. This difference was statistically significant when the data were collapsed across learning trials 2–5, F (3,174) $= 2.72$, $P < 0.05$. Pairwise comparisons showed the $E^+/\varepsilon 4^-$ group performed best overall, with the overall performance of the other 3 groups indicating that they were not different from each other statistically.

Table 3
Group means ±SD for selected CVLT scores

	ERT users		ERT non-users		
	$\varepsilon4^+$ ($n = 35$)	$\varepsilon4^-$ ($n = 66$)	$\varepsilon4^+$ ($n = 29$)	$\varepsilon4^-$ ($n = 51$)	P value
List A Trial 1	6.94(±2.57)	7.30(± 2.08)	6.76(± 2.57)	6.96(± 2.02)	0.82
List A Trial 5	11.91(± 3.22)	13.18(± 2.25)	12.62(± 2.64)	12.02(± 2.68)	0.03
Trial 5 – Trial 1	4.97(± 2.29)	5.88(± 2.34)	5.86(± 2.40)	5.06(± 2.75)	0.03
Σ Trial 1 to 5	49.91(± 13.19)	55.48(± 9.59)	50.86(± 11.52)	50.51(± 9.76)	0.08
SDFR	10.29(± 3.92)	11.83(± 2.55)	10.72(± 3.14)	10.39(± 3.12)	0.06
SDCR	11.69(± 3.37)	12.83(± 2.17)	12.03(± 3.03)	11.89(± 2.10)	0.14
LDFR	10.63(± 3.65)	12.27(± 2.65)	11.10(± 3.60)	11.22(± 2.78)	0.11
LDCR	11.51(± 3.53)	13.15(± 2.10)	11.90(± 3.38)	12.31(± 2.15)	0.15
Forgetting index:					
SDFR – LDFR	0.34(± 1.89)	0.44(± 1.52)	0.38(± 1.63)	0.82(± 2.59)	0.57
Trial5 – LDFR	−1.29(± 2.48)	−0.91(± 1.93)	−1.52(± 2.85)	−0.80(± 2.65)	0.66
Recognition:					
Hits	14.83(± 1.54)	15.15(± 1.10)	14.41(± 1.68)	14.51(± 155)	0.61
False Positivesa	1.54(± 2.15)	0.52(± 0.90)	1.14(± 1.57)	1.02(± 1.36)	0.05
DI	93.77(± 6.10)	96.94(± 3.20)	93.81(± 6.01)	94.30(± 4.39)	0.07

Note. $\varepsilon4^+$, possession of an APOE $\varepsilon4$ allele; $\varepsilon4^-$, absence of the APOE $\varepsilon4$ allele; SDFR, short delay free recall; SDCR, short delay cued recall; LDFR, long delay free recall; LDCR, long delay cued recall; DI, discriminability index.
[a]Low scores signify better performance.

3.2.2. Recall

Recall measures evaluated the ability to reproduce items presented on the original CVLT word list, either in free recall or in response to a category cue (cued recall).

3.2.2.1. Memory – Delayed recall

On the ability to recall after a few minutes' delay, 1-way ANCOVA (4 levels of the factor group) showed that there was a trend for a significant effect of group status to emerge on long delay cued recall, F (3,174) = 2.26, $P = 0.08$. Comparisons indicated that the pattern of performance across the four groups was as noted across the learning trials, with the $E^+/\varepsilon4^-$ group performing at the highest level. Similar patterns of data were revealed when the short and long delay free recall and the short delayed cued recall data were analysed. Importantly, a significant inter-group difference was most robustly observed between the $E^+/\varepsilon4^-$ and the $E^+/\varepsilon4^+$ participants.

3.2.2.2. Memory – Total recall

Total recall performance was evaluated using 1-way ANCOVA (4 levels of the factor group) by calculating the sum of recall performance across the five learning trials and four delayed trials. When this analysis was conducted, there was a similar pattern of data as previously noted across the 4 participant groups (F (3,174) = 2.50, $P = 0.06$).

Another statistical procedure was undertaken using the total recall measure: the data were filtered so that the effects of ERT could be evaluated separately in those individuals either possessing or not possessing the $\varepsilon4$ allele. When only the data from the $\varepsilon4^-$ participants was analysed using 1-way ANCOVA, there was a statistically significant effect of ERT on episodic memory performance (F (1,112) = 7.36, $P < 0.01$). However, no such effects of treatment were present if ERT was evaluated only in those individuals possessing the $\varepsilon4$ allele. These findings were also indicated when the full data set was evaluated: using 2-way (2 × 2: group × treatment) ANCOVA, there was a significant interaction between the factor of ERT status and the factor of $\varepsilon4$ status on the total number of items recalled (F (1,173) = 6.03, $P < 0.05$).

3.2.3. Recognition

3.2.3.1 Discriminability index (*DI*)

This index of recognition memory performance measures the ability to discriminate words on the target memory list from words on a similar interference list, and from distractors that were not present on either list. 1-way ANCOVA (4 levels of the factor group) showed that there was a significant difference between groups on the level of performance on the DI, F (3,174) = 4.41, $P = 0.005$. The pattern of performance across the 4 groups was similar to that previously noted with respect to the memory recall data: post hoc testing indicated significantly better performance in the $E^+/\varepsilon4^-$ group compared with the 3 other participant groups. This group difference was indicated both in terms of the number of hit and false positive responses that were

made (hits: F (3,173) = 2.19, $P = 0.09$; false positives: F (3,173) = 3.31, $P < 0.05$).

3.2.3.2. Response bias

This index of recognition performance measures an individual's tendency to respond "yes" to any item (whether correct or incorrect) on "yes"/"no" recognition. Performance on this measure did not differ significantly between the four participant groups.

Further statistical analyses were conducted in order to evaluate all the findings reported above when participants scoring either the maximum level of learning and memory performance ("ceiling") or the minimum level of learning and memory performance ("floor") were excluded from the analyses. When this was undertaken, the findings were very similar to those that have already been reported by us here.

4. Discussion

This is the first clinical study in the literature to explore systematically the interaction between oestrogen administration and APOE genotype on specific elements of learning and memory in healthy postmenopausal women. Improvements relative to age and education-matched controls have previously been observed following oestrogen supplementation in postmenopausal women on tests of learning and memory [14,15]. However, the interaction of this phenomenon with the APOE $\varepsilon 4$ allele has not yet been thoroughly investigated. The study revealed that, within a modestly sized cohort of postmenopausal women, specific components of episodic learning and memory were affected by ERT and genetic status. More specifically, oestrogen and APOE $\varepsilon 4$ status impacted upon verbal episodic memory, with the best memory performance being observed in women receiving ERT who were APOE $\varepsilon 4$ negative.

Of interest, further inspection of the memory data indicated that the most marked group difference was observed between the $E^+/\varepsilon 4^-$ and $E^+/\varepsilon 4^+$ participants. Furthermore, when the total recall data were evaluated as a composite measure of episodic memory, it was apparent that only individuals who were $\varepsilon 4^-$ benefited from ERT. This point is further emphasized by the observation that, on the vast majority of memory indices, the $\varepsilon 4^+$ group who received ERT performed no better than the $E^-/\varepsilon 4^-$ group. Taken together, these are potentially very important findings, indicating that ERT may not be able to compensate for the deleterious effects of the APOE $\varepsilon 4$ allele on memory. This finding requires further investigation, as it has substantial health implications. In particular, if there are indeed other health risks associated with ERT (such as enhanced risk of breast cancer, cardiovascular disease or thromboembolic disease), then ERT prescribed for its potential cognitive benefits may be contra-indicated in individuals who possess the APOE $\varepsilon 4$ allele.

A further important point concerns the relationship between the learning and memory data. There is a close relationship in all individuals between the ability to learn and the ability to remember, such that the former clearly affects the latter. What is striking about the data obtained in the current study is that, when one takes into account statistically the group differences in memory performance that had developed by the time of the fifth learning trial, then the observed differences in delayed recall performance across the four groups are rendered non-significant (for reasons of conciseness, these analyses are not presented in the previous section; further details are available upon request). The data obtained in this study therefore indicate that memory differences between groups (based on their ERT and $\varepsilon 4$ status) are established within a few learning trials, and these differences are then maintained across the groups in terms of delayed memory performance.

In addition, when considering the learning and memory data reported in this study, it should be noted that individual differences in performance on the CAMCOG and MMSE were controlled for within the analyses that were conducted. It should be noted that this produced a somewhat conservative analysis of the episodic memory data, given that both the CAMCOG and the MMSE contain certain elements that are related to episodic memory functioning. This consideration renders the group-related differences in learning and memory performance that we report here all the more striking.

In a related study, Yaffe et al. [19] recently found that over a 72-month period, $\varepsilon 4$ carriers manifested less of an HRT treatment effect, in that they had a faster rate of cognitive decline compared to those not possessing the $\varepsilon 4$ allele. Nevertheless, in the study reported by Yaffe et al. [19], HRT still seemed to benefit the $\varepsilon 4$ carriers when cognitive performance was compared to the $\varepsilon 4$ carriers who were not receiving oestrogen [19]. Our findings contrast with those of Yaffe et al. [19], in that, in our study, $\varepsilon 4$ carriers did not benefit from the administration of HRT, whereas those individuals who were $\varepsilon 4$ negative did. In another recent study conducted by Hays et al. [28], it was reported that the combined effects of oestrogen and progestin

did not benefit mental health or cognitive status in a large sample of postmenopausal women. However, in the study reported by Hays et al. [28], cognitive status was evaluated using a non-specific instrument derived from the MMSE. This consideration also applies to the recent study reported by Shumaker et al. [2], which used a similarly non-specific cognitive instrument. By contrast, in the present study we evaluated those specific elements of verbal episodic memory, which are known to be most sensitive to age-related cognitive decline. With respect to the discrepancies noted between the findings of this study and the findings of Yaffe et al. [19], Hays et al. [28] and Shumaker et al. [2], our findings therefore indicate that the specific elements of cognition that are being evaluated in large scale clinical trials should be carefully considered before embarking on research studies with serious and wide-ranging implications. This is an especially pertinent point in the context of those several studies that have used brief cognitive instruments such as the MMSE. The MMSE is a clinical instrument that is quick to administer, and which is very widely used, but which offers little detailed iformation and has limited cognitive specificity.

Another possible explanation for apparent discrepancies in the literature relates to the specificity, dose and temporal characteristics of the HRT that was evaluated in this study. For example, in this study unopposed ERT was investigated, as distinct from combined oestrogen-progesterone therapy, which has been evaluated in most previous studies. The contrasting effects of ERT that were observed in the $\varepsilon 4^+$ and $\varepsilon 4^-$ participants in this study do not, however, seem to be related to inadequate statistical power due to the smaller number of participants in the $\varepsilon 4^+$ group. When the $\varepsilon 4^-$ group was randomly reduced in size to make it comparable to the $\varepsilon 4^+$ group, significant effects of ERT were still observed in the $\varepsilon 4^-$ group but not in the $\varepsilon 4^+$ group. (For reasons of space, these results are not reported in this paper, but are available upon request).

The possible interaction of ERT on memory performance in those with or without the APOE $\varepsilon 4$ allele has not been properly investigated in the past. This was the central aim of our study. We evaluated verbal learning and memory, as deficits in these cognitive domains are apparent in the very early stages of AD, and these elements of cognition have been used as sensitive markers of age-related cognitive decline. Our findings indicate that ERT may be a useful prophylactic approach to age-related cognitive decline and AD in certain postmenopausal women, but its neuroprotective action may be substantially modified by possession of the APOE $\varepsilon 4$ allele. Given the potential health risks associated with the use of some forms of ERT, the findings of this paper are of considerable significance in allowing clinicians and patients to make more informed decisions regarding the prescribing of ERT in postmenopausal women.

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